reagents, wherein each offset adaptor has a recognition sequence for at least one of the second nucleic acid cleaving reagents,

(d) mixing one or more adaptor-indexers with the nucleic acid sample and covalently coupling the adaptor-indexers to the nucleic acid fragments, wherein each adaptor-indexer has a different sticky end, wherein each sticky end of the adaptor-indexers is compatible with one of the possible sticky ends that could be generated by the second nucleic acid cleaving reagents,

wherein the nucleic acid fragments to which offset adaptors and adaptor-indexers have been coupled are binary sequence tags.

REMARKS

Claims 1, 117, 121, and 126-137 are pending. Claims 1, 117, 121, and 126 are amended. Claims 2-116, 118-120, and 122-125 are canceled. Claims 127-137 are newly added. Claims 1, 117, 121, and 126 have been amended to more clearly recite applicants' invention. Claims 1, 117, 121, and 126 have been amended to recite that each sticky end or end sequence of the adaptor-indexers is compatible with "one of the possible sticky ends that could be generated by the second nucleic acid cleaving reagents." These amendments find support at least on page 40, lines 20-23. Claims 1, 117, and 126 have been amended to recite that "the first nucleic acid cleaving reagents are not type IIS restriction enzymes." These amendments find support at least on page 11, line 24, where it is indicated that Type IIS restriction enzyme need not be used, and page 16, lines 7-9, where non-Type IIS restriction enzymes are said to be preferred as the first nucleic acid cleaving reagent.

Claim 121 was amended to more clearly refer to the antecedent "offset adaptor" rather than "first offset adaptor strand." Claim 126 was amended to more clearly refer to antecedent "first offset adaptor strands" and "first adaptor-indexer strands" rather than "offset adaptors" and "adapter-indexers," respectively.

New claims 127-133 are based on original claims 1, 127-129, 117, 121, and 126, respectively, and find support at least in the original claims. Claims 127, 131, 132, and 133 also find support on page 40, lines 20-23, where compatibility of adaptor-indexer sticky ends with one of the possible sticky ends generated by nucleic acid cleaving reagents is described. Claims 127, 131, and 133 also find support at least on page 11, line 24, where it is indicated that Type IIS restriction enzyme need not be used, and page 16, lines 7-9, where non-Type IIS restriction enzymes are said to be preferred as the first nucleic acid cleaving reagent.

New claims 134 and 136 are drawn to the subject matter of original claim 1 and add steps of mixing ligator-detectors with the binary sequence tags and a detector array containing probes, covalently coupling the ligator-detectors to the probes, and detecting coupling of ligator-detectors to the detector array probes. New claims 134 and 136 find support at least in original claim 1, original claim 40 and from page 10, line 13, to page 11, line 18, where the added steps are described. Mixing and coupling can be done simultaneously (page 41, lines 18-19). New claims 135 and 137 are drawn to the subject matter of original claim 1, further requiring that the second nucleic acid cleaving reagents do not cleave in the recognition sequences of the first nucleic acid cleaving reagents. New claims 135 and 137 find support at least in claim 1 and on page 73, lines 9-25, from

page 76, line 26, to page 77, line 3, and page 81, lines 15-28, all illustrating a second cleavage outside of the recognition site of the first nucleic acid cleaving reagent

Sequence Listing

The application includes a paper copy of a Sequence Listing which is identical to the sequence listing filed in the parent application Serial No. 09/637,751. Applicant hereby requests that the computer readable form of the sequence listing submitted in application Serial No. 09/637,751, be used as the computer readable form of the sequence listing for the new application. I declare that the material on the diskette is identical to the paper copy of the Sequence Listing present in the new application, that the Sequence Listing does not add new matter to the application, and that all statements made on information and belief are believed to be true and further that these statements were made with the knowledge that willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Claims 1, 117, 121, and 126-137 are believed to be in patentable form. Actions on the merits is respectfully requested.

No fee is believed due. However, the Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 14-0629.

Respectfully submitted,

NEEDLE & ROSENBERG, P.C.

Robert A. Hodges, Ph.D.
Registration No. 41,074

Suite 1200, The Candler Building
127 Peachtree Street, N.E.
Atlanta, Georgia 30303-1811
404/688-0770

CERTIFICATE OF EXPRESS MAILING UNDER 37 C.F.R. § 1.10

I hereby certify that this correspondence is being deposited with the United States Postal Service as Express Mail, No. EL924206145US in an envelope addressed to: Box Patent Application, Assistant Commissioner for Patents, Post Office Box 2327, Arlington, VA 22202, on the date shown below.

Erick Calderon — 11/26/01
Erick Calderon — Date

Version With Markings to Show Changes Made

This application is a continuation of U.S. Application No. 09/637,751, filed August 11, 2000, which application is a continuation-in-part of U.S. Application No. 09/544,713, filed April 6, 2000, which claims the benefit of U.S. Provisional Application No. 60/148,870, filed August 13, 1999, by Paul M. Lizardi and Darin R. Latimer, entitled "Analysis of Sequence Tags With Hairpin Primers", which applications are hereby incorporated herein in their entirety by reference [. Application Serial No. 09/544,713, filed April 6, 2000, and Application No. 60/148,870, filed August 13, 1999, are hereby incorporated herein by reference].

[Figure 1 is] Figures 1A, 1B, and 1C are a listing of examples of ligator-detectors (numbered sequences) designated for use with one of two example adaptor-indexers (Figure 1A, top). The sticky end sequences (or their complements) are shown in bold.

1. (Amended) A method of producing binary sequence tags from nucleic acid fragments in a nucleic acid sample, the method comprising

(a) incubating a nucleic acid sample with one or more first nucleic acid cleaving reagents to produce nucleic acid fragments, wherein the first nucleic acid cleaving reagents are not type IIS restriction enzymes,

(b) mixing one or more offset adaptors with the nucleic acid sample and covalently coupling the offset adaptors to the nucleic acid fragments,

(c) incubating the nucleic acid sample with one or more second nucleic acid cleaving reagents to produce nucleic acid fragments with sticky ends, wherein the second nucleic acid cleaving reagents cleave at a site offset from their recognition sequence, wherein each offset adaptor has a recognition sequence for at least one of the second nucleic acid cleaving reagents,

(d) mixing one or more adaptor-indexers with the nucleic acid sample and covalently coupling the adaptor-indexers to the nucleic acid fragments, wherein each adaptor-indexer has a different sticky end, wherein each sticky end of the adaptor-indexers is compatible with [a sticky

end] one of the possible sticky ends that could be generated by the second nucleic acid cleaving reagents,

wherein the nucleic acid fragments to which offset adaptors and adaptor-indexers have been coupled are binary sequence tags.

117. (Amended) A method of producing binary sequence tags from nucleic acid fragments in a nucleic acid sample, the method comprising

(a) incubating a nucleic acid sample with one or more first nucleic acid cleaving reagents to produce nucleic acid fragments, wherein the first nucleic acid cleaving reagents are not type IIS restriction enzymes,

(b) mixing one or more first offset adaptor strands with the nucleic acid sample and covalently coupling the first offset adaptor strands to the nucleic acid fragments, wherein, after coupling, the first offset adaptor strands are fully or partially single-stranded,

(c) treating the nucleic acid sample to result in full or partial complementary sequences hybridized to the first offset adaptor strands,

(d) incubating the nucleic acid sample with one or more second nucleic acid cleaving reagents to produce nucleic acid fragments with sticky ends, wherein the second nucleic acid cleaving reagents cleave at a site offset from their recognition sequence, wherein each first offset adaptor strand has a recognition sequence for at least one of the second nucleic acid cleaving reagents,

(e) mixing one or more adaptor-indexers with the nucleic acid sample and covalently coupling the adaptor-indexers to the nucleic acid fragments, wherein each adaptor-indexer has a different sticky end, wherein each sticky end of the adaptor-indexers is compatible with [a sticky end] one of the possible sticky ends that could be generated by the second nucleic acid cleaving reagents,

wherein the nucleic acid fragments to which [offset adaptors] first offset adaptor strands and adaptor-indexers have been coupled are binary sequence tags.

121. (Amended) A method of producing binary sequence tags from nucleic acid fragments in a nucleic acid sample, the method comprising

(a) incubating a nucleic acid sample with one or more first nucleic acid cleaving reagents to produce nucleic acid fragments,

(b) mixing one or more offset adaptors with the nucleic acid sample and covalently coupling the offset adaptors to the nucleic acid fragments,

(d) incubating the nucleic acid sample with one or more second nucleic acid cleaving reagents to produce nucleic acid fragments with sticky ends, wherein the second nucleic acid cleaving reagents cleave at a site offset from their recognition sequence, wherein each [first] offset adaptor [strand] has a recognition sequence for at least one of the second nucleic acid cleaving reagents,

(e) mixing one or more first adaptor-indexer strands with the nucleic acid sample and covalently coupling the first adaptor-indexer strands to the nucleic acid fragments, wherein each first adaptor-indexer strand has a different end sequence, wherein each end sequence of the first adaptor-indexer strands is compatible with [a sticky end] <u>one of the possible sticky ends that could be</u> generated by the second nucleic acid cleaving reagents, wherein, after coupling, the first adaptor-indexer strands are fully or partially single-stranded,

wherein the nucleic acid fragments to which offset adaptors and first adaptor-indexer strands have been coupled are binary sequence tags.

126. (Amended) A method of producing binary sequence tags from nucleic acid fragments in a nucleic acid sample, the method comprising

(a) incubating a nucleic acid sample with one or more first nucleic acid cleaving reagents to produce nucleic acid fragments, <u>wherein the first nucleic acid cleaving reagents are not type IIS restriction enzymes,</u>

(b) mixing one or more first offset adaptor strands with the nucleic acid sample and covalently coupling the first offset adaptor strands to the nucleic acid fragments, wherein, after coupling, the first offset adaptor strands are fully or partially single-stranded,

(c) treating the nucleic acid sample to result in full or partial complementary sequences hybridized to the first offset adaptor strands,

(d) incubating the nucleic acid sample with one or more second nucleic acid cleaving reagents to produce nucleic acid fragments with sticky ends, wherein the second nucleic acid cleaving reagents cleave at a site offset from their recognition sequence, wherein each first offset adaptor strand has a recognition sequence for at least one of the second nucleic acid cleaving reagents,

(e) mixing one or more first adaptor-indexer strands with the nucleic acid sample and covalently coupling the first adaptor-indexer strands to the nucleic acid fragments, wherein each first adaptor-indexer strand has a different end sequence, wherein each end sequence of the first adaptor-indexer strands is compatible with [a sticky end] one of the possible sticky ends that could be generated by the second nucleic acid cleaving reagents, wherein, after coupling, the first adaptor-indexer strands are fully or partially single-stranded,

wherein the nucleic acid fragments to which [offset adaptors] first offset adaptor strands and [adaptor-indexers] first adaptor-indexer strands have been coupled are binary sequence tags.